

Claims

1. An isolated enone reductase having the following physico-chemical properties:

- a) Molecular mass:  $61,300 \pm 5,000$  Da  
(Consisting of one subunit having a molecular mass of  $45,000 \pm 5,000$  Da),
- b) Co-factor: NADPH and NADH,
- c) Substrate specificity: active on  $\alpha$ ,  $\beta$ -unsaturated ketones,
- d) Optimum temperature: 55 - 60°C at pH 7.4,
- e) Optimum pH: 4.5 - 8.5.

2. An enone reductase according to claim 1, which is derived from a microorganism which is capable of producing the enone reductase having the properties as defined in claim 1.

3. An enone reductase according to claim 2, wherein the microorganism is a yeast.

4. An enone reductase according to claim 2, wherein the microorganism is *Candida kefyr* (*Candida macedoniensis*) IFO 0960, its functional equivalents, subcultures, mutants or variants.

5. A process for producing an enone reductase having the following physico-chemical properties:

- a) Molecular mass:  $61,300 \pm 5,000$  Da  
(Consisting of one subunit having a molecular mass of  $45,000 \pm 5,000$  Da),
- b) Co-factor: NADPH and NADH,
- c) Substrate specificity: active on  $\alpha$ ,  $\beta$ -unsaturated ketones,
- d) Optimum temperature: 55 - 60°C at pH 7.4,
- e) Optimum pH: 4.5 - 8.5.

which process comprises cultivating a microorganism, which is capable of

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producing the enone reductase having the above properties, in an aqueous nutrient medium under aerobic conditions, disrupting the cells of the microorganism, and isolating and purifying the enone reductase from said extract.

6. The process according to claim 5, wherein the microorganism is a yeast.

5 7. A process for producing levodione from ketoisophorone which process comprises contacting ketoisophorone with

(i) an enone reductase having the following physico-chemical properties:

a) Molecular mass:  $61,300 \pm 5,000$  Da  
(Consisting of one subunit having a molecular mass of  $45,000 \pm 5,000$

10 Da),

b) Co-factor: NADPH and NADH,

c) Substrate specificity: active on  $\alpha$ ,  $\beta$ -unsaturated ketones,

d) Optimum temperature: 55 - 60°C at pH 7.4,

e) Optimum pH: 4.5 - 8.5.

15 in the presence of NADH or NADPH; or

(ii) cells or a cell-free extract of a microorganism belonging to the genus Candida, capable of producing the enzyme as defined in (i),

and isolating the resulting levodione from the reaction mixture.

8. The process according to claim 7, wherein the microorganism is a yeast.

20 9. The process according to claims 7 or 8, wherein the reaction is conducted at a pH in the range of from 4.5 to 8.5.

10. The process according to any one of claims 7 to 9, wherein the temperature of the reaction is in the range of from 30 to 60°C.

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